Swine Plasma Immunoglobulins for Prevention and Treatment of Post-Weaning Diarrhoea

Optimizing Stability Towards Gut Conditions

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SWINE PLASMA IMMUNOGLOBULINS FOR PREVENTION AND TREATMENT OF POST-WEANING DIARRHOEA: OPTIMIZING STABILITY TOWARDS GUT CONDITIONS

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Background

Post-weaning diarrhoea (PWD) is a common condition in intensive swine production, resulting in reduced welfare of weaners and economic losses for the farmer as a result of illness, death, treatment costs, e.g. high consumption of antibiotics and zinc oxide.

Aim

1. **Developing feed additives for oral provision for protection against PWD based on natural antibodies (immunoglobulins) derived directly from inexpensive raw materials.**
2. **To increase stability (reducing gut proteolysis) by cross-linking the immunoglobulins (Igs).**

Conclusions

- **The optimal conditions for Igs-multimerisation were observed to at pH 9 using 5-10 mM $NaIO_4$, which confers to increased reactivity towards Salmonella Diarizonae after pepsin digestion.**
- **These results suggest that cross-linked Igs could be used for prevention/treatment of PWD and reduce antibiotic consumption.**

Materials & Methods

Immunoglobulin isolation:

Porcine Igs were purified from blood plasma at UpFront Chromatography A/S (Copenhagen) by high-volume Expanded Bed Adsorption with a proprietary adsorbent. Plasma was obtained from a Danish slaughter house. The immunoglobulins were multimerised by controlled periodate oxidation of immunoglobulin-bound carbohydrate (Fig. 1). The multimerisation process was stopped by increasing pH to 12. Cross-coupled Ig-species were analysed by non-reduced 12% Bis-Tris SDS PAGE or gel filtration (S300 Sephacryl). Complexes were either visualised by silver staining or Western blotting; primary antibody: biotinylated mouse anti-pig Fc antibody (BD, clone F007-1241); developed by alkaline phospatatse-streptavidin and NBT/BCIP.

Figure 1: Sodium Periodate ($NaIO_4$) multimerisation

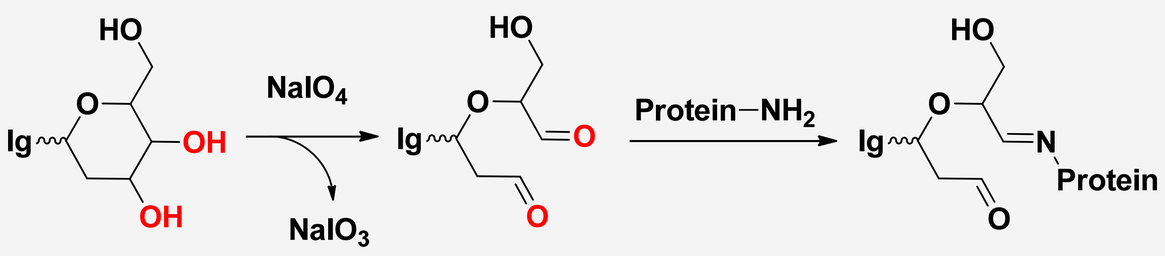


Figure 1. Carbohydrates on immunoglobulins, comprise diols (in red), which are cleavable by the periodate generating aldehydes that in turn can bind to free amines on the polypeptide chain of other immunoglobulins.

ELISA:

For testing the reactivity of the swine Igs on pathogenic bacterial antigens a competitive ELISAs were applied. Along with the swine Igs either Genway Biotech's anti-E. coli (18-511-245057) or anti-salmonella (18-511-245055) HRP-conjugated antibodies were used. Initially, antigens were coated in the wells before a mix of swine Igs and HRP-conjugated antibody was added. The read out was dependent on the ability of the swine Ig to inhibit the signal by interfering with the binding of conjugated antibody to its ligands.

In vitro (piglet) stomach conditions:

According to Petschow & Talbott (J ped gastroent nutr. 1994) the stomach pepsin concentration is 13 units/ml; this was mixed with swine Igs and incubated in 50mM sodium acetate pH 3 for 3 hrs. at 37°C where after the pepsin was inactivated by increasing the pH to 9.6 by adding Na_2CO_3.

Results

IMMUNOGLOBULIN MULTIMERISATION:

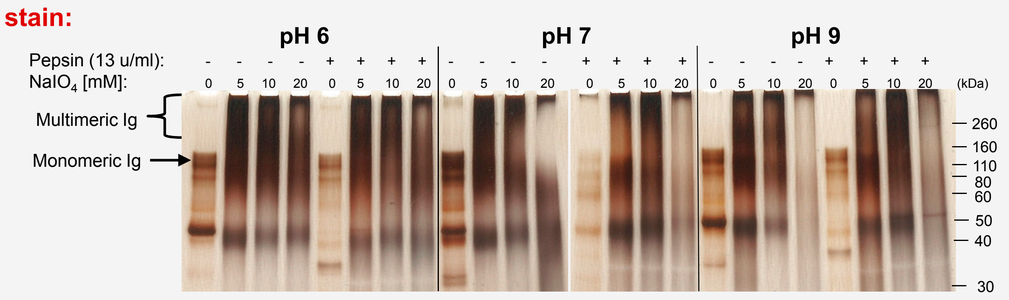
The degree of Igs-multimerisation was tested at 5, 10, and 20 mM $NaIO_4$ and at different pH values (6, 7 and 9), and all conditions subjected to $NaIO_4$ oxidation resulted in multimerisation (Fig. 2A+B). As the increasing multimerisation was associated with lower signal on Western blot (developed with anti-porcine Fc-antibody) suggests that the Fc moieties are situated in the centre of the complex shielded from the anti-Fc-antibody (Fig. 2A, Western blot). The lower level of protein in the samples multimerised with 20 mM $NaIO_4$ might be due to aggregated Igs caught during filtration of the samples preceding gelfiltration (Fig. 2B, 20 mM).

$NaIO_4$-multimerisation seems to sacrifice some Ig-reactivity (Fig. 2C, 5 mM, pH 9) but on the other hand gain some reactivity by size and complexity (Fig. 2C, 10-20 mM).

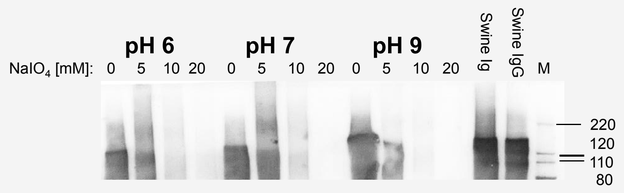
Figure 2:

A: SDS PAGE (non-reduced)

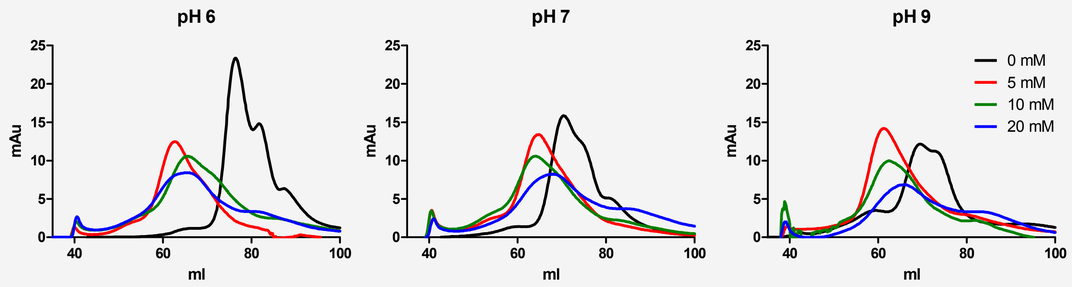
Silver stain:



Western blot:



B: Gelfiltration



C: Competitive ELISA

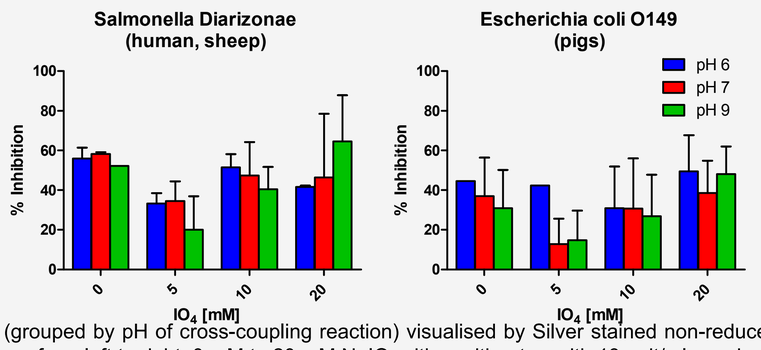


Figure 2. (A): The samples (grouped by pH of cross-coupling reaction) visualised by Silver stained non-reduced SDS PAGE. In each pH-group $NaIO_4$ increases from left to right: 0 mM to 20 mM $NaIO_4$ either without or with 13 unit/ml pepsin. Western blot was visualised by a biotinylated mouse monoclonal anti-porcine Fc antibody proceeded by alkaline phosphatase streptavidin. **(B):** Different periodate concentrations (coloured lines) as compared to non-periodate aggregates (black line) on Sephacryl S300 gelfiltration. **(C):** Swine IgG reactivity to bacterial antigens measured in a competitive ELISA. The results are indicated as the degree of inhibition of the conjugated anti-Salmonella or anti–E. coli antibodies by the swine Igs.

GUT CONDITIONS:

The pepsin concentration applied was not strong enough to digest the non-multimerised nor the multimerised swine Igs (Fig. 2A). By comparing the different levels of inhibition between the digested and non-digested samples, in the competitive ELISA, it appears that Igs multimerised at 5 mM $NaIO_4$ gain an increased ability to inhibit binding of the conjugated antibody (Fig. 3), thus multimerisation at pH 9 and 5 mM could preferable.

Figure 3:

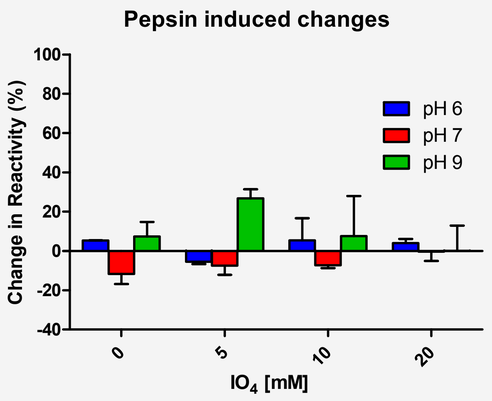


Figure 3. Pepsin digestions of the porcine Igs. Pepsin-digested and non-digested samples were mixed 1:1 with HRP-conjugated anti-salmonella antibody and incubated in wells coated with salmonella antigens. Based on the degree of inhibition of the HRP-signal, any changes between the non-digested and digested samples were recorded.